MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Intended purpose

The *ampli*Cube Coronavirus SARS-CoV-2 is a qualitative *in-vitro* test to detect the RNA of SARS coronaviruses (SARS-CoV) (screening) and to specifically detect the RNA of the novel SARS-CoV-2 (confirmation) from human sputum, swabs, BAL (bronchoalveolar lavage), tracheal secretions or stool.

2 Field of application

SARS coronaviruses such as SARS-CoV-2 (causative pathogen of the COVID-19 pandemic) spread primarily via droplets in exhaled air to transmit from person to person. Symptoms range from fever, cough and dyspnoea to pneumonia and acute respiratory distress syndrome and ultimately death in persons with comorbidities.
The *ampli*Cube Coronavirus SARS-CoV-2 is used for screening and specific detection (confirmation) of SARS-CoV-2.

3 Test principle

The test is a real-time RT (reverse transcriptase) PCR system. It uses specific primers and labelled probes for transcription of RNA into cDNA and amplification and detection of the RNA of SARS-Beta-CoV/Sarbeco and SARS-CoV-2.
To ensure that the nucleic acids isolated from the patient sample do not contain any substances that inhibit RT-PCR, an internal control (IC) is added to the sample during the nucleic acid isolation. This IC is transcribed into cDNA, amplified and detected in the same PCR batch. This eliminates false negative test results due to inhibition of the RT-PCR reaction. At the same time, the IC serves as evidence of nucleic acid extraction from the patient sample.
Probes to specifically detect the pathogen-specific nucleic acids are marked with the reporter dyes FAM (SARS-Beta-CoVSarbeco: E-Gen) and HEX (SARS-CoV-2: ORF1a) and the probes for detecting the internal control are marked with ATTO 647N. This allows simultaneous detection of all target sequences in a single reaction mixture. The FAM-marked probe detects SARS coronaviruses. Differentiating SARS-CoV-2 is carried out in the HEX channel.
The Ct value (cycle threshold) describes the part of the curve in which the fluorescence rises exponentially above the background value for the first time.

4 Reagents

4.1 Package contents

The reagents in one pack are sufficient for 50 (500) assays.
Each set of reagents contains:

P&P MIX	**150 µl (1x 1500 µl)** Primer & Probe mix for SARS-Beta-CoV/Sarbeco, SARS-CoV-2 and internal control (**green lid**)
ENZYME	**600 µl (4x 1500 µl)** enzyme mix (**white lid**) Contains reverse transcriptase and DNA polymerase. (**Component is stained blue.**)
CONTROL INT	**250 µl (2x 1250 µl)** internal control **(transparent lid)**
CONTROL +	**170 µl (1x 1700 µl)** positive control **(red lid)**
CONTROL -	**2x 1800 µl (8x 1800 µl)** negative control (**blue lid**)
INSTRU	**1** instructions for use

4.2 Additionally required reagents, materials and equipment

- Depending on the used real-time PCR-Cycler, MIKROGEN provides reagents for the colour compensation: MIKROGEN *ampli*Cube Color Compensation (LightCycler® 480 II (Roche) article no. 50502), MIKROGEN *ampli*Cube Color Compensation (cobas z 480 Analyzer (Roche), article no. 50503) or colour calibration kit for CFX96 (Bio-Rad, article no. 50505). If processing at Mic (bms) PCR cycler, MIKROGEN provides Mic assay-templates.
- Nucleic acid extraction: The following nucleic acid extraction systems are recommended: MagNA Pure® System, Total Nucleic Acid Isolation Kit (Roche) or *alpha*Clean Mag RNA/DNA Kit (MIKROGEN) with processing on the M32, M48 or M96 extractor (Biocomma)
- Real-time Cycler: LightCycler® 480 II (Roche), cobas z 480 Analyzer (Roche), CFX96 (Bio-Rad), QuantStudio 5 (Applied Biosystems), Mic (bms), Rotor-Gene Q (Qiagen)
- 96-well PCR plates and films or reaction tubes (PCR-clean): follow the recommendations of the manufacturer of the real-time PCR cycler
- Micropipettes with single-use tips with filter 10 µl, 20 µl, 100 µl and 1000 µl
- Vortex mixer with high rotational speed (recommended 3200 rpm)
- Mini centrifuge
- If necessary, plate centrifuge
- PBS or H_2O *(PCR grade)* if using flocked nylon fibre swabs with no transport medium
- NaCl (physiological, 0.9%) using the extraction-free Fast protocol
- Single-use gloves, powder-free
- Cooling block

5 Shelf life and handling

- Store reagents between -25 °C and -18 °C before and after use.
- Repeated thawing and freezing of the components (more than ten times) must be avoided. It is recommended to aliquot the test components after the first thawing.
- Always appropriately chill reagents during the working steps (+2 °C to +8 °C).
- Keep the kit components away from direct sunlight throughout the test procedure.
- Before starting the test, all reagents must be completely thawed, mixed (briefly vortex) and centrifuged.
- The packages have an expiry date, after which no further guarantee of quality can be given.
- The test must only be performed by trained, authorised and qualified personnel.
- Substantial changes made by the user to the product or the directions for use may compromise the intended purpose of the test specified by MIKROGEN.
- Cross-contamination can lead to false test results. Add patient samples and controls carefully. Ensure that the reaction mixtures are not transferred to other wells.

6 Warnings and safety precautions

- Only use for *in-vitro* diagnostics.
- All patient samples must be treated as potentially infectious.
- Suitable single-use gloves must be worn throughout the entire test procedure.
- All reagents and materials that come into contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentrations and incubation times specified by the manufacturer must be followed.
- Do not replace or mix the reagents with reagents from other kit batches, other MIKROGEN PCR kits or with reagents from other manufacturers.
- Read through and carefully follow all instructions before performing the test. Deviations from the test protocol described in the instructions for use can lead to false results.

7 Sampling and preparation of reagents

7.1 Sample material and sample preparation

The starting material for the *ampli*Cube Coronavirus SARS-CoV-2 is RNA extracted from sputum, swabs, BAL, tracheal secretion or stool of human origin. The quality of the nucleic acid preparation affects the test result. It must be ensured that the extraction method selected is compatible with the real-time PCR technology.

7.1.1 *Sample preparation*

When using a flocked nylon fibre swab without transport medium:
1. Incubate the swab in 0.5 ml PBS (for extraction using the MagNA Pure® system) or 0.5 ml H_2O (for extraction using *alpha*Clean Mag RNA/DNA) for 5 minutes at room temperature.
2. Discard the swab and use the PBS or H_2O suspension for the nucleic acid extraction.

7.1.2 *Sample preparation without prior nucleic acid extraction*

An extraction-free processing is described in chapter 11.4 Fast protocol.

7.2 Extraction of nucleic acids

Extract the nucleic acids from the patient sample and the negative control (NC). We recommend a starting volume for the extraction of 200 µl and an elution volume of 50 µl or 100 µl depending on the extraction system. Extractions (MagNA Pure® System (Roche)) from 400 µl starting material eluted into 100 µl showed comparable results. Follow the instructions from the manufacturer of the extraction kit.

1. Thaw the internal control (IC) (transparent lid) and the negative control (NC) (blue lid).
   Ensure that the IC and the NC are completely thawed. Mix the IC and the NC before use by briefly vortexing and then briefly centrifuge.
2. For the extraction, add 5 µl IC to each patient sample and the NC. The IC should be added to the sample/lysis buffer mix and not directly to the sample material
3. Extract the patient sample and the NC. (Note: the NC cannot be used in the PCR without extraction.)
4. The positive control is not extracted.

The following nucleic acid extraction system is recommended and was used for the performance assessment:

Extraction system	Sample volume	Elution volume
MagNA Pure® 24 (Roche) Total NA Isolation Kit	200 µl	50 µl
M96 Nucleic Acid Extraction System (biocomma) *alpha*Clean Mag RNA/DNA Kit (MIKROGEN)	200 µl	100 µl

If you would prefer to use other extraction methods, please contact the manufacturer to clarify the compatibility.

7.3 Preparing the master mix

1. Thaw the Primer & Probe mix (green lid) and the enzyme mix (white lid). Protect the reagents from light while doing so.
   Ensure that the reagents are completely thawed. Mix the reagents before use by vortexing and then briefly centrifuge.
2. Prepare the master mix using the following pipetting scheme:

Components	Master mix for 1 reaction
Primer & Probe mix	3 µl
Enzyme mix	12 µl
Total volume	15 µl

3. Mix the entire master mix by vortexing and then briefly centrifuge.
4. Produce 15 µl master mix for each PCR reaction.

7.4 Preparing the RT-PCR reaction

1. Thaw the positive control (PC) (red lid).
   Ensure that the reagents are completely thawed. Mix the reagents before use by vortexing and then briefly centrifuge.

Components	1 reaction
Master mix from 7.3	15 µl
Sample eluate or eluate of the NC or the PC	10 µl

2. Pipette 10 µl of the sample eluate into 15 µl master mix.
3. Pipette 10 µl of the positive control (not prepared) into 15 µl master mix.
4. Pipette 10 µl of the eluate of the negative control into 15 µl master mix.

Every run must include a positive and a negative control.
Seal the PCR plate with an optically clear adhesive film or seal the reaction tube with the lid provided.

⚠ **The PCR plates or test tubes must be vortexed for at least 5 sec at maximum speed and then briefly centrifuged.**

⚠ **PCR test tubes for the Mic PCR cycler must be vortexed for at least 10 sec at maximum speed.**

8 Programming the real-time cycler

The *ampli*Cube Coronavirus SARS-CoV-2 was evaluated with the LightCycler® 480 Instrument II (Roche) and validated at cobas z 480 Analyzer (Roche), CFX96 (Bio-Rad), QuantStudio 5 (Applied Biosystems), Mic (bms) and Rotor-Gene Q (Qiagen).

8.1 Setting the detection channels

LightCycler® 480 Instrument II (Roche)

	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Colour	Green	Yellow	Red
Reporter dye	FAM	HEX	ATTO 647N
Excitation	465 nm	533 nm	618 nm
Emission	510 nm	580 nm	660 nm

For the LightCycler® 480 II, it is necessary to first use the Color Compensation kit (article no. 50502), which is provided by MIKROGEN.

cobas z 480 Analyzer (Roche)

	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Colour	Green	Yellow	Red
Reporter dye	FAM	HEX	ATTO 647N
Excitation	465 nm	540 nm	610 nm
Emission	510 nm	580 nm	670 nm

For the cobas z 480 Analyzer (Roche), it is necessary to first use Color Compensation (article no. 50503), which is provided by MIKROGEN.

CFX96 (Bio-Rad)

	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Colour	Green	Yellow	Red
Reporter dye	FAM	HEX	ATTO 647N
Mode	all channels		

The data acquisition and evaluation at CFX96 is carried out using the method *calc / data acquisition mode: all channels*. A dye calibration of the CFX96 (Bio-Rad) must be carried out in advance for ATTO 647N, which is provided by MIKROGEN (CFX96 (Bio-Rad) dye calibration set, article no. 50505)

QuantStudio 5 (Applied Biosystems)

	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Colour	Green	Yellow	Red
Reporter dye	FAM	HEX	ATTO 647N
Excitation	X1 / 470 nm	X2 / 520 nm	X5 / 640 nm
Emission	M1 / 520 nm	M2 / 558 nm	M5 / 682 nm
Quencher	[none]	[none]	[none]

Choose under settings 1. Run mode „standard“, 2. Reference dye “none”, 3. Experiment type “custom”.

Mic (bms)

	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Colour	Green	Yellow	Red

MIKROGEN provides you with validated Mic assay-templates for processing. Please use exclusively MIKROGEN Mic assay-templates. The current MIKROGEN Mic assay-templates are freely available for download on the MIKROGEN homepage (www.mikrogen.de).

Rotor-Gene Q (Qiagen)

	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Colour	Green	Yellow	Red
Reporter dye	FAM	HEX	ATTO 647N
Excitation	470 nm	530 nm	625 nm
Emission	510 nm	555 nm	660 nm
Gain	5	5	5

8.2 PCR program

Reverse transcription	50 °C	8 minutes
Denaturation	95 °C	3 minutes
Amplification	**45 cycles**	
• Denaturation	95 °C	10 seconds
• Annealing/elongation	60 °C	45 seconds

Essential information about programming the different real-time cyclers can be found in the instructions for the cycler used. For specific information about programming the real-time PCR cycler when using the *ampli*Cube Coronavirus SARS-CoV-2, please contact the manufacturer.

9 Results

The data analysis on the LightCycler® 480 II is carried out using the *Abs Quant/2nd Derivative Max* method.

9.1 Validation

1. The negative control must be below the threshold. The internal control (IC) must have a positive curve for the negative control. If the negative control has a positive curve (contamination) or if the IC is not valid in the negative control, the test run cannot be analysed.
2. The positive control must have a positive curve.
   The Ct value for the positive control must be < 33. A positive control outside this range indicates that there is a problem with the amplification.
3. The internal control (IC) must have a positive curve for negative samples. The signal for the IC for a patient sample must be compared to the signal of the IC in the extracted negative control. A difference of > +3 for the Ct value of the IC of a sample compared to the IC of the negative control or the absence of an IC signal for the sample may indicate significant inhibition of the RT-PCR reaction. In these cases a negative test result is invalid.

9.2 Evaluation

The data can be analysed with the corresponding PCR cycler software or a software solution for automated PCR analysis and interpretation specifically supported by MIKROGEN. Using a LightCycler® 480 II the analysis can be carried out either using the *Abs Quant/2nd Derivative Max* method (recommended) or the *Abs Quant/Fit Points* method. Additional information and corresponding instructions are available from MIKROGEN upon request.

		SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Colour		Green	Yellow	Red
Reporter dye		FAM	HEX	ATTO 647N
Evaluation with	**LightCycler® 480**	465-510	533-580	618-660
	cobas z 480	465-510	540-580	610-670
	CFX96	FAM	HEX	Cy5
	QuantStudio 5	FAM	VIC	ATTO 647N
	Mic	FAM	HEX	Cy5
	Rotor-Gene Q	green	yellow	red

Signals above the threshold are evaluated as positive results. Empty fields in the table are considered negative results.

Colour	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)	Internal control (IC)
Green	Positive	---	---
Yellow	---	Positive	---
Red	---	---	Positive*

The following test results are possible:

SARS-Beta-CoV/ Sarbeco (E Gene) (green)	SARS-CoV-2 (ORF1a) (yellow)	Internal control (IC) (red)	Possible interpretations for SARS-CoV-2
Positive	---	Positive*	**Potentially positive!** Nucleic acids specific for SARS coronaviruses found in the examined material; no confirmation of SARS-CoV-2 **Sample should be extracted and analysed again or a new patient sample requested!**
Positive	Positive	Positive*	**Positive!** Nucleic acids specific for SARS coronaviruses found in the examined material; confirmation of SARS-CoV-2
---	Positive	Positive*	**Potentially positive!** No nucleic acids specific for SARS coronaviruses found in the examined material but nucleic acids specific for SARS-CoV-2 detected **Sample should be extracted and analysed again or a new patient sample requested!**
---	---	Positive*	**Negative!** No nucleic acids specific for SARS-CoV and SARS-CoV-2 found in the examined material
---	---	---	**Invalid! Cannot be evaluated!**

* In the case of positive signals in the detection channels for the pathogens, the signal for the internal control is not required for the test interpretation. A high pathogen load in the patient sample can lead to a reduced or missing signal for the internal control.

10 Limits of the method, restrictions

- Test results must always be viewed in the context of the clinical situation. Therapeutic consequences of the findings must be linked to the clinical data.
- A negative *ampli*Cube Coronavirus SARS-CoV-2 test result cannot rule out infection with the particular pathogens.

11 Performance characteristics

11.1 Diagnostic sensitivity and specificity

The sensitivity and specificity were determined using defined positive and defined negative samples. The panel of positive SARS-CoV-2 samples consisted of wild-type samples, Variants of Concern samples (Alpha, Beta, Gamma, Delta, Omicron) and other SARS-CoV-2 variants.

Table 1: Defined SARS-CoV-2 positive samples

*ampli*Cube Coronavirus SARS-CoV-2	**SARS-Beta-CoV/Sarbeco (E Gene) / SARS-CoV-2 (ORF1a)** (n = 190)
Negative	1
Positive	189*
Sensitivity [%]	**99.47**
CI [%] Confidence interval	**97.08 – 99.91**

* Samples that only showed a positive amplification curve in the E Gene or ORF1a were also considered positive (interpretation: "potentially positive"). In this collective, this was 4/189 (2.1%).

Table 2: Defined SARS-CoV-2 negative samples

*ampli*Cube Coronavirus SARS-CoV-2	**SARS-Beta-CoV/Sarbeco (E Gene) / SARS-CoV-2 (ORF1a)** (n = 540)
Negative	533
Positive	7*
Specificity [%]	**98.70**
CI [%] Confidence interval	**97.35 – 99.37**

* Samples that only showed a positive amplification curve in the E Gene or ORF1a were also considered positive (interpretation: "potentially positive"). In this collective, this was 5/7 (71.4%).

11.2 Analytical sensitivity

11.2.1 *Limit of Detection (LoD) in copies/PCR*

The limit of detection (LoD) of the *ampli*Cube Coronavirus SARS-CoV-2 was determined using a dilution series of gBlock DNA of known concentration on a LightCycler® 480 II System (Roche). The 95% limit of detection was determined using probit regression analysis with CombiStats™ Version 5.0 software (Council of Europe). The limit of detection was then confirmed with 20 replicates at the LoD.

Table 3: Limit of detection (LoD) in copies/PCR

	SARS-Beta-CoV/Sarbeco (E Gene, screening)	**SARS-CoV-2 (ORF1a, confirmation)**
LoD [copies/PCR] 95% limit of detection	1.53	2.23
95% CI [copies/PCR] confidence interval	0.82 – 4.29	1.34 – 5.84

11.2.2 *Limit of Detection (LoD) SARS-CoV-2 in International Units*

In addition, the LoD for the SARS-CoV-2 target regions was determined with three independent dilution series (7 dilution levels each) using the WHO standard (First WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)) including nucleic acid extraction (*alpha*Clean Mag RNA/DNA Kit on M96 Biocomma) on a LightCycler® 480 II system (Roche) and subsequent probit regression analysis (CombiStatsTM version 6.0 software (Council of Europe)). The detection limit of the *ampli*Cube Coronavirus SARS-CoV-2 is given in International Units (IU) per µl WHO standard.

Table 4: Limit of detection (LoD) for SARS-CoV-2 in IU/µl WHO standard

	SARS-Beta-CoV/Sarbeco (E Gene, screening)	**SARS-CoV-2 (ORF1a, confirmation)**
LoD [IU/µl] 95% limit of detection	4.52	3.24
95% CI [IU/µl] confidence interval	2.28 – 12.64	1.66 – 8.72

11.3 Analytical specificity

The BLAST trial (www.ncbi.nlm.nih.gov/blast/) shows that the selected primers and probes of the *ampli*Cube Coronavirus SARS-CoV-2 specifically detect the selected pathogens.
Furthermore, the specificity was determined by studying genomic DNA/RNA of other human pathogenic bacteria and viruses.

Table 5: Bacteria and viruses that were tested in order to demonstrate the analytical specificity of the *ampli*Cube Coronavirus SARS-CoV-2.

Bacteria
Bordetella pertussis
Bordetella parapertussis
Campylobacter coli
Campylobacter jejuni
Chlamydia pneumoniae
Haemophilus influenzae
Klebsiella pneumoniae
Legionella pneumoniae
Legionella longbeachae
Legionella micdadei
Moraxella catarrhalis
Mycoplasma pneumoniae
Pneumococci
Pseudomonas aeruginosa
Salmonella enterica
Staphylococcus aureus
Streptococcus pneumoniae
Yersinia enterocolitica

Viruses
Coronavirus MERS
Coronavirus hCoV
Enterovirus
Influenza A
Influenza A H1N1
Influenza A H3N2
Influenza A avian
Influenza A H7N9
Influenza B
Parainfluenzavirus
Respiratory syncytial virus (RSV) A
Rhinovirus 58 (A)

None of these samples generated a positive signal. The primers and probes used in the *ampli*Cube Coronavirus SARS-CoV-2 showed no cross-reactions with the pathogens listed in Table 5. The internal control (IC) was valid in all tests.

11.4 Fast protocol (without RNA/DNA extraction)

Extraction-free processing with physiological sodium chloride solution (NaCl, 0.9%) and dry swabs.

1. Incubate the swab in 0.4 ml of physiological sodium chloride solution for 5 minutes at room temperature.
2. Discard the swab and use 10 µl of the washout directly for PCR.
3. Please note the following change when preparing the master mix:

Table 6: Preparing the master mix

Components	**Master mix for 1 reaction**
Primer & Probe mix	3 µl
Enzyme mix	12 µl
Internal control (IC)	**2 µl**
Total volume	17 µl

4. Mix the entire master mix by vortexing and then briefly centrifuge.
5. Produce 17 µl master mix for each PCR reaction.

Table 7: Preparing the RT-PCR reaction

Components	**1 reaction**
Master mix (see table 6)	17 µl
Washout solution of the sample or NaCl as NC or PC	10 µl

Do NOT use the negative control (NC) provided in the kit as the negative control, use NaCl instead.

⚠ **The PCR plates or test tubes must be vortexed for at least 5 sec at maximum speed and then briefly centrifuged.**

⚠ **PCR test tubes for the Mic PCR cycler must be vortexed for at least 10 sec at maximum speed.**

Setting the detection channels and PCR program see under chapter 8.1 and 8.2.

Validation: See under chapter 9.1 for the negative control (NC) and positive control (PC).
ATTENTION: By adding the internal control (IC) to the master mix, a signal for the internal control appears in addition to the signal of the positive control in the red channel. The signal of the internal control in the positive control is not evaluated. The internal control (IC) must have a positive curve for negative samples.

Diagnostic sensitivity and specificity (Fast protocol):
The performance of the Fast protocol was determined by testing 123 nasal and throat swabs (Copan, no. 159C). Each of these 123 samples was partly spiked with inactivated, viral SARS-CoV-2 particles (concentration: 3.5 particles / µl washout, used standard vircell, MBTC030) and a part of the washout solution was directly added to the PCR (= negative sample collective). A sample was considered positive if at least one of the two parameters (E Gene or ORF1a) was detected. The mean values of the Ct values were 32.8 for the E Gene, 35.3 for the ORF1a gene and 26.6 for the internal control (LightCycler® 480 II (Roche)). One sample gave an invalid negative result (0.8%) due to an invalid internal control. The diagnostic sensitivity and specificity (3.5 particles / µl washout) were 99.2% and 100%, respectively.

Table 8: Results of 122 nasal and throat swabs with extraction-free processing (invalid sample n = 1, is not shown).

Fast protocol *ampli*Cube Coronavirus SARS-CoV-2	**SARS-Beta-CoV/Sarbeco (E Gene) / SARS-CoV-2 (ORF1a)** (n = 122)
Positive	121
Sensitivity [%]	**99.2**
Negative	122
Specificity [%]	**100**

11.5 Equivalence of different sample material

The coefficient of variation (CV) was determined for the Ct value between water and the extract of the particular sample material after the addition of plasmid DNA of known concentration.

Table 9: Equivalence of different sample material

	SARS-Beta-CoV/Sarbeco (E Gene)	SARS-CoV-2 (ORF1a)
CV [%] (BAL, H_2O)	1.09	1.59
CV [%] (sputum, H_2O)	1.19	1.90
CV [%] (swab, H_2O)	0.72	0.99
CV [%] (stool, H_2O)	0.78	2.19

The coefficient of variation (CV), based on the Ct value (cycle threshold) between water and the nucleic acid extracts (obtained for the various sample materials), was ≤ 2.19 % for all target genes.

12 Literature

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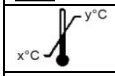
7. Ute Eberle, Clara Wimmer, Ingrid Huber, Antonie Neubauer-Juric, Giuseppe Valenza, Nikolaus Ackermann, Andreas Sing (for the Bavarian SARS-CoV-2-Public Health Laboratory Team), Comparison of nine different commercially available molecular assays for detection of SARS-CoV-2 RNA, European Journal of Clinical Microbiology & Infectious Diseases, Springer, published online: 29 January 2021, https://doi.org/10.1007/s10096-021-04159-9

We will be pleased to send you additional literature on request.

13 Explanation of symbols

	Content is sufficient for <n> formulations Number of formulations
P&P MIX	Primer & Probe mix
ENZYME	Enzyme mix
CONTROL INT	Internal control
CONTROL +	Positive control
CONTROL -	Negative control
INSTRU	Instructions for use
	Follow the instructions for use
CONT	Contents, contains
IVD	*In-vitro* diagnostic medical device
LOT	Batch number
REF	Order number
	Use by Expiry date
	Store between x °C and y °C
	Manufacturer

14 Manufacturer and version data

***ampli*Cube Coronavirus SARS-CoV-2**	Article no. **50143 (50144)**
Instructions for use Valid from	GAACCVS003EN 2022-05

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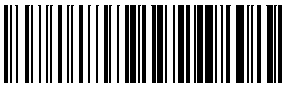

GAACCVS003